Docket #: S20-209

Total RNA Profiling of Biological Samples and Single Cells

Researchers at Stanford and CZ Biohub San Francisco have developed a method capable of assaying a broad spectrum of coding and noncoding RNA from a single cell, thus enabling simultaneous analysis of protein-coding, long-noncoding, microRNA and other noncoding RNA transcripts from single cells.

Characterizing the transcriptional state of single cells has primarily focused on protein-coding RNA. However, a growing number of studies indicate that noncoding RNAs (ncRNAs) are actively involved in cell function and specialization. In addition, protein-coding RNA is transcribed from only 1-2% of the genome, whereas ncRNA covers ~70% of the genomic content and the majority of all cellular transcripts. The role of these transcripts in shaping different cell types and states remains poorly understood. Current techniques aimed at measuring ncRNA in single cells can only target a certain subset of noncoding transcripts, and no current method can simultaneously quantify all RNA types within a cell. Thus, the ability to map the regulatory connection between coding and noncoding transcripts within a cell is limited. This gap motivates the need for a novel single cell technology capable of assaying both poly(A)+ and poly(A)- RNA, irrespective of transcript length.

Stage of Development
Research –

in vitro

Stage of Research
The inventors developed a "one-pot" scalable method designed to capture both coding and noncoding transcripts regardless of their length. This method, named Smart-seq-total, harnesses the template-switching capability of MMLV reverse transcriptase to generate full-length cDNA with high yield and quality. Smart-seq-total captures nonpolyadenylated RNA through template-independent addition of

poly(A) tails, and further oligo-dT priming of all cellular transcripts, meaning all RNA molecules can also be tagged with unique molecular identifiers (UMIs). SMART-seq-total thereby quantifies mRNA and other types of RNA in the same cell.

Technology Reference
CZB-170S-PC, S20-209

Applications

- The annotation of cell types and states based on integration of mRNA data with other existing scRNA-seq datasets.
- The discovery of non-coding regulatory patterns of the respective cell types and states.

Advantages

- The sensitivity of Smart-seq-total estimated based on external RNA control consortium capture is comparable to Smart-seq2.
- Smart-seq-total detects a broader spectrum of RNA types than previous single-cell approaches.
- Smart-seq-total allows the incorporation of UMIs for absolute quantitation into both short and long RNA molecules.

Publications

- Isakova, A., Neff, N., Quake, S.R. "Single-cell quantification of a broad RNA spectrum reveals unique noncoding patterns associated with cell types and states." PNAS (2021), 118(51), e2113568118.

Patents

- Published Application: WO2021236963
- Published Application: 20230193254

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